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CHORIOCARCINOMA CELL LINE RESPONSE TO DEXAMETHASONE

ODGOVOR HORIOKARCINOMSKIH ĆELIJSKIH LINIJA NA TRETMAN DEKSAMETAZONOM

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Summary: Choriocarcinoma cell lines JAr and JEG-3 are model systems for the study of transformed trophoblast. Both cell lines were shown to produce galectin-1, expression of which was increased in choriocarcinoma when compared to the normal trophoblast of pregnancy. In this study the effects of synthetic glucocorticoid dexamethasone were investigated in both JAr and JEG-3 cell lines by the MTT test, cell based ELISA, and the cell adhesion and migration tests. Viable cell number/cell proliferation of JAr cells was significantly increased after treatment with 0.1 and 1 nmol/L of dexamethasone, while proliferation of JEG-3 cells was significantly increased after treatment in the whole concentration range of dexamethasone (0.1–100 nmol/L). Galectin-1 in JAr cells was modulated by dexamethasone, which mildly, but significantly decreased production at low concentrations (0.1 and 1 nmol/L). In JEG-3 cells production of galectin-1 was significantly decreased only after treatment with 100 nmol/L of dexamethasone. Cell adhesion of JEG-3 was significantly increased in the presence of lactose, an inhibitory sugar for gal-1, while dexamethasone induced decrease of JEG-3 cell migration. These findings have shown that dexamethasone may affect proliferation, gal-1 production and cell migration, in a cell line specific manner. These data suggest that glucocorticoid treatment *in vivo* might have the potential to affect cell functions in choriocarcinoma.

Keywords: choriocarcinoma cell lines, dexamethasone, proliferation, galectin-1

Kratak sadržaj: Horiokarcinomske ćelijske linije JAr i JEG-3 su model sistemi za ispitivanje transformisanog trofoblasta. Obe ćelijske linije eksprimiraju galektin-1, čija je povećana ekspresija pokazana u horiokarcinomima u odnosu na normalni trofoblast trudnoće. U ovom radu ispitivan je efekat sintetskog glukokortikoida deksametazona na JAr i JEG-3 ćelijske linije, upotrebom MTT testa, ELISA testa na ćelijama i testovima adhezije i migracije. Broj živih ćelija, kao indikator proliferacije ćelija, značajno je povećan nakon tretmana 0,1 i 1 nmol/L deksametazonom, a proliferacija JEG-3 je značajno povećana u čitavom opsegu upotrebljenih koncentracija deksametazona (0,1–100 nmol/L). Galektin-1 je u JAr moduliran deksametazonom, koji blago, ali značajno smanjuje produkciju galektina-1 u niskim koncentracijama (0,1 i 1 nmol/L). Kod JEG-3 ćelija, produkcija galektina-1 je značajno smanjena samo nakon tretmana 100 nmol/L deksametazonom. U prisustvu laktoze kao inhibitornog šećera za galektin-1 adhezija JEG-3 ćelija je značajno povećana, dok deksametazon uz to smanjuje i migraciju JEG-3 ćelija. Rezultati dobijeni u ovom radu pokazuju da deksametazon utiče na proliferaciju i u manjoj meri na galektin-1 kod JAr i JEG-3 ćelija, na način specifičan za ćelijsku liniju. Dobijeni podaci ukazuju na to da bi tretman glukokortikoidima *in vivo* mogao imati uticaja na ćelije horiokarcinoma.

Ključne reči: horiokarcinomske ćelijske linije, deksametazon, proliferacija, galektin-1

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List of Abbreviations: BSA, bovine serum albumin, CELISA, cell based enzyme linked immunosorbent assay, DEX, dexamethasone, FCS, fetal calf serum, gal-1, galectin-1, GC, glucocorticoid, GR, glucocorticoid receptor, HRP, horseradish peroxidase, mgal-1, monoclonal anti-galectin-1 antibody, MTT, thiazolyl blue, pgal-1, rabbit polyclonal anti-galectin-1 antibodies, PDGF, platelet-derived growth factor, RT, room temperature, TGF-β, transforming growth factor-β, TMB, 3, 3', 5, 5' tetramethyl benzidine.

Introduction

Choriocarcinoma is a malignancy characterized by abnormal proliferation and transformation of trophoblastic cells of the human placenta. This neoplasm is almost exclusively composed of multinucleated syncytiotrophoblast and mononuclear cytotrophoblast (1). To study the mechanisms responsible for the changes underlying tumor progression, a number of choriocarcinoma cell lines were established. Among them BeWo, JAr and JEG-3 are the most widely used (2, 3). Although the cells of each of these cell lines differ in their morphology, all have an invasive phenotype, proliferate (2, 3) and express galectin-1 (gal-1) (4, 5). This soluble lectin with an affinity for β-galactoside residues is expressed by various normal and pathological tissues (6). In cancer, gal-1 could be involved in cell transformation, apoptosis, regulation of cell cycle, cell adhesion, migration and inflammation. Expression or overexpression of gal-1 has been well documented in many diverse tumors, including those arising from the thyroid, endometrium, colon, prostate, bladder, pancreas and ovary (7). It has been shown that the expression of gal-1 is increased in the transformed trophoblast of choriocarcinoma, and this finding could be related to the increased invasiveness of the transformed phenotype (8).

Glucocorticoids (GCs) are frequently used as co-treatment before, during and after chemotherapy. Because of the pro-apoptotic and anti-proliferative effects in lymphoid tissues, GCs, such as the highly effective synthetic agent dexamethasone (DEX), are widely used as co-medication in cancer therapy (9). The distinct effects of GCs on different tumor types have been reported. Contrary to the supportive action of GCs in the anti-tumor therapy of lymphomas, glucocorticoid-induced resistance toward cancer therapy is common in ovarian, lung and cervical carcinomas (9, 10). A possible involvement of GCs in the regulation of gal-1 expression in choriocarcinoma cells has not been studied so far. However, treatment with DEX influenced production of gal-1 and the process of cell invasion *in vitro* in the HTR-8/SVneo extravillous trophoblast cell line (11). It has also been shown that the invasion of first-trimester trophoblast cell line HTR-8/SVneo was sensitive to lactose, an inhibitory sugar for the lectin-type interaction of galectins (11). Therefore, the aim of this study was to investigate whether DEX might affect cell proliferation, adhesion and migration of JAr and JEG-3 cells, as well as their production of gal-1.

Materials and Methods

Materials

Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 Ham (DMEM/F12) with and without phenol red, Minimum Essential Medium Eagle's (MEM Eagle's) without phenol red, antibiotic/antimycotic mixture (100 mg/mL streptomycin sulfate, 100 units/mL penicillin G and 25 μg/mL amphotericin B), thiazolyl blue (MTT), bovine serum albumin (BSA) and DEX were from Sigma (Chemical Co., USA). Fetal calf serum (FCS) was obtained from PAA Laboratories (Linz, Austria). MEM Eagle's culture media with phenol red were obtained from the Institute of Immunology and Virology (Belgrade, Serbia). Horseradish peroxidase (HRP) and 3, 3', 5, 5' tetramethyl benzidine (TMB) were from ICN Biochemicals (Aurora, Ohio, USA). Growth factor reduced Matrigel™ and laboratory plasticware were obtained from Falcon (Becton Dickinson, USA) and Costar (Corning Incorporated, NY, USA). Other chemicals were p.a. grade.

Antibodies

Antiserum to human placental gal-1 was produced in our laboratory as described earlier (4, 12). Monoclonal anti-gal-1 antibody (mgal-1) was from NovoCastra Laboratories (Newcastle upon Tune, UK). Sheep anti-rabbit IgG-HRP conjugate was produced in our laboratory according to the method of Nakane (13).

Cell culture

JEG-3 choriocarcinoma cells from the European Collection of Cell Cultures (ECACC, Salisbury, UK) were cultured in MEM Eagle's media, containing 10% FCS and antibiotic/antimycotic mixture (complete medium). JAr choriocarcinoma cells (ATCC, USA) were cultured in DMEM/F12 supplemented with 10% FCS and antibiotic/antimycotic mixture. Cortisol and progesterone concentrations in FCS used to culture cells were measured by commercial kits (RIA, INEP, Belgrade, Serbia) and were below the level of detection.

Treatment with DEX

JEG-3 and JAr cells were seeded in 96-well plates at $2x10^4$cells/well in respective media and cultured in humidified 5% CO_2/95% air at 37 °C. The medium was removed after 24 h and cells were rinsed once with 0.05 mol/L phosphate buffered saline (PBS), pH 7.2. Cells were incubated in the respective medium with DEX (at final concentrations of 0.1, 1, 10, and 100 nmol/L) and without DEX (control group). After 48 h, confluent cells were washed once with PBS and fixed with ice-cold methanol/acetone (1:1) at room temperature (RT) for 10 min, dried and stored at -20 °C until use.

Cell based ELISA

Cell based ELISA was performed as described previously (11). Gal-1 in control and DEX treated JAr

and JEG-3 was detected with rabbit polyclonal anti-gal-1 antibodies (pgal-1). Bound antibodies were detected by HRP conjugated sheep anti-rabbit-IgG. For the assessment of non-specific binding, nonimmune rabbit IgG was used in place of anti-gal-1.

Determination of viable cell number/cell proliferation (MTT assay)

Choriocarcinoma JAr and JEG-3 cells were plated in 96-wells ($2x10^4$ cells/well). After treatment with DEX, the MTT assay was performed as recently described (11, 14).

Adhesion assay

The adhesion assay was a modification of a previosly described procedure (15). Ninety-six-well plates were precoated with 250 μg/mL of Matrigel for 1 h at 37 °C, treated with 50 μL of 3% BSA to block non-specific binding sites, washed with PBS and used in the adhesion assay. The trypsinized JAr and JEG-3 cells were resuspended at $2.5x10^5$ cells/mL in the respective serum-free media with 0.1% BSA. Cells were preincubated with 1 nmol/L DEX, 100 mmol/L lactose, pgal-1 (120 μg/mL) or medium (control group) for 1 h in humidified 5% CO_2/95% air at 37 °C with occasional agitation and plated in pre-coated 96 wells at $2.5x10^4$ cells/well. After incubation for 1 h, the plates were rinsed once gently with PBS. The attached cells were stained using 50 μL of 0.4% crystal violet in 10% ethanol and 1% ammonium oxalate for 5 min. The excess of dye was removed by immersing the plates in water and drying at RT. The incorporated crystal violet was dissolved in 100 μL/well of 33% glacial acetic acid and optical density was read at 540 nm.

Migration assay

The migration assay was a modification of a previously described protocol (16). Matrigel was diluted to 1 mg/mL in serum-free DMEM/F12 or MEM Eagle's medium. The undersides of transwell inserts (8 μm pore size) were coated by immersion in Matrigel solution and incubation for 1 h at RT. JEG-3 and JAr cells ($5x10^4$) were seeded onto inserts in 200 μL of complete MEM Eagle's or DMEM/F12 medium in the presence of: a) DEX (1 nmol/L), b) lactose (100 mmol/L) and c) mgal-1 (40 μg/mL). Culture medium (500 μL) supplemented with the same concentrations of DEX, lactose, or mgal-1 was added to the lower chambers. Controls were grown in the respective media without supplements. After incubation for 24 h in a humidified atmosphere at 37 °C, media were removed, and the inner side of insert was wiped with a swab, to remove cells which had not migrated through the membrane. The outer side of the membrane was gently rinsed 2 times with PBS. Migrated cells (attached to the membrane or surface of the plate) were stained using 250 μL of 0.4% crystal violet dye. After 10 min, crystal violet was eluted with 200 μL of 33% glacial acetic acid/well, and 100 μL of aliquots were transferred from each well to the 96-well plate. Absorbance was read at 540 nm.

Data analysis

Values expressed as percent of control are given as mean ± SD. Statistical analysis of the data was carried out with the Statistical Software Program version 5.0 (Primer of Biostatistics) using one-way ANOVA with values considered significantly different when $p<0.05$.

Results

Cell proliferation

A possible effect of treatment with DEX (0.1–100 nmol/L) on the viable cell number, i.e. proliferation of JAr and JEG-3 cells was studied. Dexamethasone was found to influence the cell number, as determined by the MTT assay (*Figure 1*). The viable cell number of JAr cells was significantly increased in the lower concentration range of DEX (0.1 and 1 nmol/L). Upon treatment with 10 and 100 nmol/L DEX, the cell number of JAr cells was slightly but not significantly increased. On the other hand, the viable cell number of JEG-3 cells was significantly increased to approximately 120% of control after treatment in the whole concentration range of the hormone (0.1–100 nmol/L DEX).

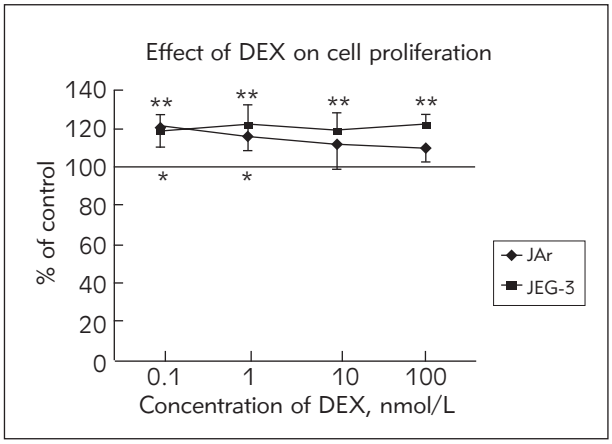

Figure 1 Effect of DEX on viability of JAr and JEG-3 cells. Cells were incubated without (control) or with DEX (0.1–100 nmol/L). Values are given as percentage of the control (means±SD); n=12. Differences vs. control significant at $p<0.05$ (*), $p<0.005$ (**).

Gal-1 protein expression in cells treated with DEX

The effect of DEX (0.1–100 nmol/L) on gal-1 production in JAr and JEG-3 cells was investigated using the choriocarcinoma cell based ELISA (CELISA) test. Relative levels of gal-1 were determined in DEX treated vs. control wells, normalized for the observed effects of DEX on JAr and JEG-3 cell proliferation, and expressed as a percentage of the control value (*Figure 2*). In JAr cells, 0.1 and 1 nmol/L of DEX significantly decreased the levels of gal-1 by 17%. The higher concentrations of DEX (10 and 100 nmol/L) induced a small, but not significant decrease in gal-1. Treatment of JEG-3 cells with 0.1–10 nmol/L DEX induced no difference in gal-1 production. The highest concentration of DEX (100 nmol/L), however, significantly decreased gal-1 production to 89% of the control value.

Cell adhesion and migration in choriocarcinoma cell lines JAr and Jeg-3

A possible influence of gal-1 interactions on the processes of cell adhesion and migration of the studied choriocarcinoma cell lines was investigated. Both lectin and non-lectin type interactions were studied using either inhibition by lactose or a specific antibody. *Figure 3A* illustrates the effects of lactose (100 mmol/L), pgal-1 (120 μg/mL) and DEX (1 nmol/L) on the adhesion of JAr and JEG-3 cells. Lactose significantly increased the adhesion of JEG-3 cells to Matrigel by up to 118% ($p<0.05$), compared to control. Under the same experimental conditions, no effect, however, was observed in the adhesion of JAr cells. Treatment with polyclonal anti-gal-1 antibodies induced no significant change in the adhesion of JAr and JEG-3 cells. Cell adhesion in either cell line was not altered upon treatment with DEX (*Figure 3A*).

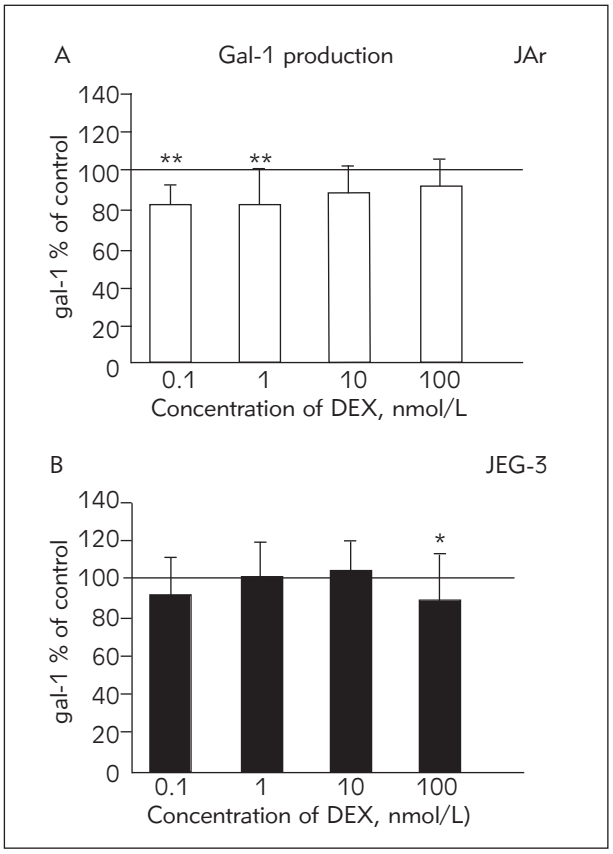

Figure 2 Effect of DEX on gal-1 in choriocarcinoma cell lines. Gal-1 protein in JAr (A) and JEG-3 (B) cell lines after treatment with DEX (0.1–100 nmol/L). Cells were maintained for 48 h in respective medium without (control) or with DEX. Data (means±SD) were corrected for the cell number after each experiment. Differences vs. control significant at $p<0.05$ (*), $p<0.005$ (**), n=15.

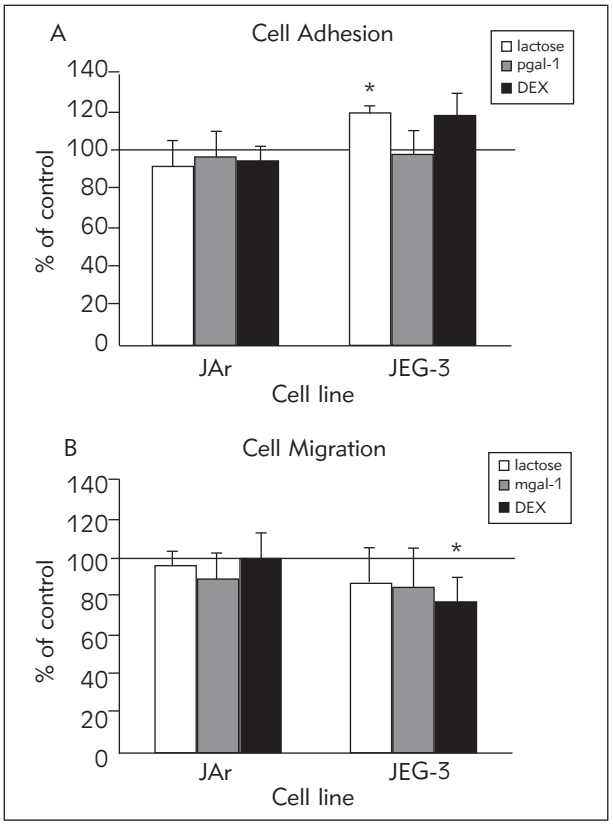

Figure 3 A) Effect of lactose (100 mmol/L), pgal-1 antibodies (120 μg/mL) or DEX (1 nmol/L) on JAr and JEG-3 cell adhesion. The cell adhesion assay was performed using Matrigel™ coated 96-well plates. Data (means±SD) are presented as percentage of control, differences vs. control significant at $p<0.05$ (*), n=6. B) Effect of lactose (100 mmol/L), mgal-1 antibody (40 μg/mL) or DEX (1 nmol/L) on JAr and JEG-3 cell migration. The cell migration assay was performed using inserts. Data (means±SD) are presented as percentage of control, differences vs. control significant at $p<0.05$ (*), n=7.

The influence of the same treatments on transmembrane cell migration was studied. Migration of JAr and JEG-3 in the respective media containing lactose (100 mmol/L), mgal-1 (40 μg/mL) or DEX (1 nmol/L) was compared to the corresponding control (*Figure 3B*). Lactose induced a small, but not significant inhibition of migration in both choriocarcinoma cell lines. Similarly, the monoclonal antibody against gal-1 slightly decreased migration of both JAr and JEG-3 cells. Significant decrease of migration by 23% has only been observed after incubation of JEG-3 cells with DEX. No effect, however, was observed in the migration of JAr cells under the same experimental conditions.

Discussion

There is ample evidence in the literature that links galectins to diverse malignancies. Previously reported data show that the increased or altered expression of gal-1 in tumors could be considered a sign of malignant tumor progression that is often related to metastasis (17). The glucocorticoid DEX, which is widely used as co-medication in cancer therapy, was found critical in the regulation of gal-1 in the neonatal lung, at the level of transcription, translation and degradation (18, 19) and was shown to regulate the production of gal-1 in normal trophoblast cells (11). However, there is no information available regarding the regulation of gal-1 production in the transformed trophoblast. Therefore, the aim of this study was to investigate the possible effects of DEX on cell proliferation, gal-1 expression, adhesion and migration in JAr and JEG-3 choriocarcinoma cells. Both cell lines were shown as appropriate models for this study because they proliferate in culture, show invasive behavior and have uniform and strong expression of gal-1 (3, 5).

The effects of GCs are generally exerted through the glucocorticoid receptor (GR). The glucocorticoid receptor has been identified in primary trophoblast cells, as well as in the extravillous trophoblast cell line HTR-8/SVneo (20, 21). It has been shown that one of the cell lines used in this study, JEG-3, expresses GR, while JAr cells were reported to lack the GR (22, 23). However, an independent study has shown that some JAr cells contain low levels of GR, and therefore could be sensitive to glucocorticoid treatment (24).

Since the DEX effects on gal-1 production were determined in this study over a 48 h interval, it was important to establish whether this GC influences cell proliferation. The concentrations of DEX used have already been shown to affect synthesis of extracellular matrix proteins in terms of trophoblast and gal-1 in HTR-8/SVneo cells (11, 25, 26). The data obtained over a wide range of concentrations (0.1–100 nmol/L) in this study showed that DEX had a stimulatory effect on proliferation in both cell types. In this study an increase of up to 20% was observed in JEG-3 cells in the whole concentration range of DEX studied, and up to approximately 20% in JAr cells at lower concentrations, while the higher doses of DEX induced a slight, but not significant increase in viable cells. Similar effects on cell proliferation were reported for OVM, M130, OAW-42, SKOV-3, GG ovarian carcinoma cells, as well as for freshly isolated primary ovarian carcinoma cells upon treatment with DEX (9).

There is little information available regarding the regulation of gal-1 protein. Several molecules, such as TGF-β, PDGF, angiotensin II, hepatocyte growth factor, have been discussed as possible modulators of gal-1 production (27, 28). Our previous results demonstrated that gal-1 protein in HTR-8/SVneo cells was modulated by DEX treatment. The effect was dose-dependent, as gal-1 was increased after treatment with 0.1–10 nmol/L DEX, and slightly reduced at 100 nmol/L (11). The data obtained in this study suggested that gal-1 protein production in JAr and JEG-3 choriocarcinoma cell lines differed regarding susceptibility to glucocorticoid treatment. Gal-1 in JAr cells was reduced in the whole range of the used DEX concentrations, significantly only at 0.1 and 1 nmol/L DEX. On the other hand, gal-1 in JEG-3 cells was decreased at 100 nmol/L DEX only. The data presented here indicate that gal-1 of the transformed trophoblast can be modulated by GCs, in a cell line specific manner, possibly due to the different levels of GR expressed and/or state of differentiation of JAr and JEG-3 choriocarcinoma cells.

Gal-1 is involved in the multistep process of tumor metastasis, which includes changes in cell adhesion, increased cell migration and invasiveness (7). It has been shown previously that expression of gal-1 is increased in choriocarcinoma (8), which may suggest an implication of this lectin in the pathology of trophoblast. However, the precise mechanisms have not been elucidated so far. We have reported here preliminary data regarding the potential involvement of gal-1 and DEX in the adhesion and migration of choriocarcinoma cells. The presence of lactose in culture media, as an inhibitory sugar for gal-1 lectin activity, mildly increased the adhesion of JEG-3 cells. However, no influence on JEG-3 cell adhesion was observed with addition of polyclonal antibodies against gal-1. The antibody available for this study was not reported to block function, which may explain the absence of effect. With respect to the differences observed between the two studied cell lines, the presence of another member of galectin family – galectin-3, with shared carbohydrate specificity in JEG-3 cells (29), may explain the difference in the effects of lactose and polyclonal antibodies against gal-1 on JEG-3 cell adhesion. On the other hand, DEX reduced the migration of JEG-3 cells, at the same time increasing the viable cell number. A similar inhibitory effect of DEX on cell migration and invasion of U373 ME glioblastoma cells was reported (30).

In conclusion, it was shown here that the synthetic GC dexamethasone influenced cell proliferation, and to a lesser extent gal-1 production, in both studied choriocarcinoma cell lines. Dexamethasone may also affect cell migration in a susceptible cell line such as JEG-3. These data suggest that GC treatment *in vivo* may have a potential to affect choriocarcinoma cells, which however warrants further investigations.

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